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Effect of Pemetrexed combined with cis−platinum chemotherapy on matrix metalloproteinase VEGF, NK cells and immune function in patients with non−squamous non−small cell lung cancer

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ABSTRACT

Objective: To explore effect of Pemetrexed combined with cis-platinum chemotherapy on matrix metalloproteinase (MMPs), vascular esandothelial growth factor (VEGF), NK cells and immune function in patients with non-squamous non-small cell lung cancer. **Method:** A total of 86 cases of non-squamous non-small cell lung cancer patients were divided into control group (n=44) and observation group (n=42), control group was given docetaxel combined cis-platinum chemotherapy, pemetrexed combined cis-platinum chemotherapy, was applied for observation group. Compared MMP-2, MMP-9, VEGF, NK cells and immune function level before and after treatment in both groups. **Results:** MMP-2, MMP-9, VEGF, NK cells, $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ level in both groups before treatment was no significant difference. After treatment, MMP-2, MMP-9, VEGF, CD8+level in both groups was significant lower than before treatment intra-group, and observation was lower than control group, there was significant difference. After treatment, NK cells, $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ level in both groups was increased dramatically than before treatment of intra-group, moreover, NK cells, $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$level in observation group after treatment was obvious higher than in control group after treatment, there was significant difference. **Conclusion:** Pemetrexed combined with cis-platinum chemotherapy for non-squamous non-small cell lung cancer could effectively decrease serum MMPs, VEGF level and increase NK cell level, regulate immune function, with definite clinical significance.

1. Introduction

Non-squamous non-small cell lung cancer usually occurred in elderly patients, a kind of common malignant cancer in clinic, its lethality rate and morbidity was the first in all cancers. Once it was diagnosed, usually was in terminal stage, which had lost operation opportunity. At present, clinical treatment mainly contained radiotherapy, chemotherapy and Chinese Traditional Medicine. However, due to multiple patients were in end-stage, therefore, chemotherapy was considered to be optimum scheme[1,2]. MMP-2 and MMP-9 highly expressed in tumor, promoted tumor extracellular matrix degradation and basal membrane destroy[3]. Vascular endothelial growth factor and body immune function played an important role in cancer development process[4]. This research was aimed to investigate effect of pemetrexed combined with cis-platinum chemotherapy on above biochemical indexes.

2. Research object and method

2.1. General data

Selected 86 cases of non-squamous non-small cell lung cancer patients who were admitted in our hospital from June 2015 to February 2017 as objects. All patients were conformed to diagnostic standard of non-squamous non-small cell lung cancer and all of them were first time to accept chemotherapy, no patients using immunopotentiator. Divided patients into observation group (n=42) and control group (n=44) according to random number table. In control group, 28 males, 16 females; aged from 51 to 78 years old; including 23 cases of III stage, 21 cases of IV stage; In observation

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Table 1.
Comparison of matrix metalloproteinase level before and after treatment.

Group	*n*	Treatment time	MMP-2 (ng/mL)	MMP-9 (ng/mL)
Control group	44	Before treatment	131.47±10.69	1 883.75±325.17
		After treatment	92.26±5.71*	1 033.89±251.64*
Observation group	42	Before treatment	130.19±11.52	1 891.28±350.19
		After treatment	73.24±6.25*#	896.87±190.61*#

Note: compared with indexes before treatment of intra-group, *P<0.05; compared with indexes of control group after treatment, #P<0.05.

group, 28 males, 14 females; aged from 50 to 79 years old; including 20 cases of III stage, 22 cases of IV stage. The gender and age data of both groups was close, the difference was no statistical significant (P>0.05), being comparable. All patients excluded severe cardiovascular disease, combined with other complication. This research was conformed to related standard of hospital ethics committee, all patients and its family members was informed and participated in treatment willingly.

2.2. Treatment method

Observation group was given pemetrexed combined with cis-platinum chemotherapy: one time intramuscular injection of Vitamin B12 was given for patients before giving pemetrexed treatment 7 d (Produced by Shandong Fangming Pharmaceutical Co. Ltd, Approval number: H37021054), 1mg/time, in later, given one time intramuscular injection of Vitamin B12 every 3 period; given oral folic acid tablets before pemetrexed treatment 7 d or at least 5 d (Produced by Hangzhou AoYiPollen Pharmaceutical Co. Ltd, Product lot number: 20150421), took drug in the whole treatment period, drug withdrawal after last time of pemetrexed treatment 21 d; given 4 mg of oral hexadecadrol on the day and before and after giving pemetrexed (Guangdong Sancai Shiqi Pharmaceutical Co. Ltd, Product lot number: 20150502), two times/day; intravenous drip of pemetrexed that added 0.9% sodium chloride injection into 500 mg/m^2 diluted to 100 mL, intravenous drip time exceeded 10 min at the first day in treatment period, after pemetrexed drug withdrawal, given cis-platinum (Qilu Pharmaceutical Co. Ltd, Product lot number: 20150409) intravenous drip, added 5% glucose injection into 75 mg/m^2, drip after diluting time exceeded 2 h, given hydrated and diuresis treatment. In the period of treatment, patients were given drug for stopping vomiting. The control group was given docetaxel combined cis-platinum chemotherapy, added 75 mg/m^2 of docetaxel (Zhejiang Haizheng Pharmaceutical Co. Ltd, Approval number H20093092) into 0.9% sodium chloride to 250 mL, finished instillation in one hour after sufficiently mixing. In the same time, patients were given cis-platinum and docetaxel treatment method was same as observation group. A chemotherapy period was 21 d, at least 2 periods.

2.3. Detection index

Detected 3-5 mL of fasting periphery venous blood of patients before and after treatment two periods, divided into 2 tubes, one tube was for centrifugation and obtain supernatant, in order to detected MMP-2, MMP-9 AND VEGF level by ELISA method; after heparin anti-coagulation, the other tube was for detection of immune function CD3$^+$, CD4$^+$, CD8$^+$, CD4$^+$/CD8$^+$ T-cell and NK cell level by flow cytomerty.

2.4 Statistical method

Statistical Software SPSS 17.0 was used for research raw data processing and analyzing, all the indexes were in accord with normal distribution, representing methods was (Mean ± SD), t-test was applied to comparison of two sample averages intra-group before and after treatment and interblock, P<0.05 indicated the difference was statistical significant.

3. Results

3.1. Comparison of matrix metalloproteinase level before and after treatment

Before treatment, comparison of MMP-2, MMP-9 level in both groups was no statistical significant (P>0.05). After treatment, MMP-2, MMP-9 level in observation group was (73.24±6.25) ng/mL, (896.87±190.61) ng/mL respectively, significantly lower than control group. After treatment, this two levels were (92.26±5.71) ng/mL, (1 033.89±251.64) ng/mL, the difference was significant (P<0.05). Moreover, MMP-2, MMP-9 level in both group before and after treatment was decreased significant than intra-group before treatment, the difference was significant (P<0.05). Result was shown in Table 1.

3.2 Comparison of VEGF and NK cell level in both groups before and after treatment

Before treatment, VEGF and NK cell level in both groups was close, the difference was not significant (P>0.05). After treatment, VEGF level in control group and observation group was (396.05±38.66) ng/L and (255.13±35.15) ng/L, was lower than intra-group before treatment (P<0.05), moreover, VEGF level in observation group was lower than control group, there was significant statistical difference (P<0.05); after treatment NK cells level in observation group was (13.91±2.86)%, was increased dramatically compared with control group that was (12.23±2.14)%, the difference was significant

Table 2.
Comparison of VEGF and NK cell level in both groups before and after treatment.

Group	n	Treatment time	VEGF (ng/L)	NK cell (%)
Control group	44	Before treatment	741.1±49.27	11.61±1.07
		After treatment	396.05±38.66*	12.23±2.14*
Observation group	42	Before treatment	730.98±48.82	11.59±1.13
		After treatment	255.13±35.15*#	13.91±2.86*#

Note: compared with indexes before treatment of intra-group, *P<0.05; compared with indexes of control group after treatment, #P<0.05.

Table 3.
Comparison of immune function index before and after treatment.

Group	n	Treatment time	$CD3^+$ (%)	$CD4^+$ (%)	$CD8^+$ (%)	$CD4^+/CD8^+$
Control group	44	Before treatment	55.59±5.14	26.83±4.48	35.98±6.52	1.02±0.34
		After treatment	60.56±5.47*	33.66±5.37*	31.68±5.15*	1.27±0.45*
Observation group	42	Before treatment	55.38±5.24	26.94±4.42	35.82±6.54	1.03±0.38
		After treatment	64.98±5.67*#	41.65±5.83*#	24.17±4.32*#	1.37±0.31*#

Note: compared with indexes before treatment of intra-group, *P<0.05; compared with indexes of control group after treatment, #P<0.05.

(P<0.05), moreover, NK cell level in both groups after treatment was higher than intra-group before treatment, the difference was statistical significant (P<0.05), as shown in Table 2.

3.3. Comparison of immune function index before and after treatment

Before treatment, T lymphocyte level in observation group and control group was close, the difference was not statistical significant (P>0.05). After treatment, $CD3^+$, $CD4^+$, $CD4^+/CD8^+$level in observation group was (64.98±5.67)%, (41.65±5.83)% and (1.37±0.31) that was increased obviously compared with control group after treatment that was (60.56±5.47)%, (33.66±5.37)% and (1.27±0.45), the difference was significant (P<0.05); moreover $CD3^+$, $CD4^+$, $CD4^+/CD8^+$ level in both groups after treatment was higher than intra-group before treatment, difference was statistical significant (P<0.05); $CD8^+$ level in observation group and control group after treatment was (31.68±5.15)% and (24.17±4.32)% respectively, which was significantly lower than that intra-group before treatment, moreover, $CD8^+$ level in observation group after treatment was significant lower than control group, difference was significant (P<0.05). As shown in Table 3.

4. Discussion

Lung cancer was a kind of malignant tumor whose lethality rate was highest in the worldwide, non-squamous non-small cell lung cancer was one of the most common in all of lung cancer pathological type, accounted for 90% of all lung cancer cases, and male patients were more than female patients[5,6]. Pathological stage of 50% patients with non-squamous non-small cell lung cancer in treatment belonged to III stage or appeared transferring, had lost operation opportunity. In recent a lot of researches had found that chemotherapy based on platinum was one of the most effective methods for treating non-squamous non-small cell lung cancer[7,8]. Cis-platinum and carboplatin was anti-cancer drug that widely applied in clinic, its efficacy was definite and cytotoxicity was strong. More and more studies had demonstrated that as a kind of multiple target point anti-metabolism drug, pemetrexed was a thymidlate symthase/dihydrofolate reductase double inhibitor that was able to inhibit synthesis of pyrimidine and purine from multiple approaches, thereby achieved anti-cancer effect, and improved total survival rate of non-squamous cancer[11]. Related researches had indicated that docetaxel could selectively inhibit T lymphocyte function and regulate immune function[12,13]. Efficacy of docetaxel combined with platinum chemotherapy in patients with non-small lung cancer have been demonstrated that was used as treatment method in control group, in order to explore effect of pemetrexed combined with cis-platinum chemotherapy on MMP-2, MMP-9, VEGF and immune function.

Integrity of extracellular matrix and basal membrane was basis of preventing infiltration and transfer from tumor cell to healthy tissue and cell. At present, relationship of tumor metastasis and anginogenesis was researched widely, and pointed out that VEGF played an important role in matrix vessel formation, enhancement of its level could improve formation of new vessels, in addition, contributed to tumor cell falling off and promoted all kinds of proteases that secreted by tumor cells into blood circulation, accelerated metastasis of tumor cells at some degree[14,15]. In tumor development, combination of tumor cell and extracellular matrix surface receptor would secrete directly or induce host cell produced a mass of MMPs, which was critical proteins that was for extracellular matrix and basilar membrane solution. High level of expression revealed high tumor invasive ability and malignant degree[16]. MMP-2 and MMP-9 were important proteins of MMPs family, related research found that MMP-2 and MMP-9 level was related to tumor maximum diameter and lympthatic metastasis, and pointed out that highly expression could trigger tumor necrosis, enhanced infiltration of tumor cell to periphery healthy tissue and cell, promoted tumor metastasis[17,18]. This research results revealed that two chemotherapy schemes could effectively decrease MMP-2, MMP-9 and VEGF level, moreover improvement degree of pemetrexed combined with cis-platinum chemotherapy was superior. The results indicated that pemetrexed combined with cis-platinum chemotherapy lowered MMP-2, MMP-9 level validly, thereby reduced the degeneration of extracellular matrix and basal membrane, decreased VEGF level, in order to inhibit tumor vessel formation and decrease invasion and metastasis.

NK cell was natural killer cell which was able to kill directly some tumor cells and virus infected cell. Its quantity reduced or activity decreased could weaken killing and purge function of body to tumor cell, led to anti-cancer ability decrease[19]. Cellular immunity and humoral immunity participated in tumor defense and elimination, and that immune function based on T cell was extremely key. T lymphocyte level could reveal immune function condition[20]. Compared with healthy people, $CD3^+$, $CD4^+$ T cell and NK cell reduced, $CD8^+$ T cell increased, $CD4^+/CD8^+$ level significantly decreased in patients with cancer, revealed T lymphocyte and NK cell immunocomppromise[21,22]. This research indicated that patients T lymphocyte and NK cell level was improved effectively after pemetrexed combined with cis-platinum chemotherapy, illustrating immune microenvironment was optimized and immune system was reconstructed, this was good to body monitor tumor cell. Results indicated that the reason why the chemotherapy could enhance survival rate of patients was related to its effect on immune function. In conclusion, pemetrexed combined with cis-platinum chemotherapy could reduce MMPs, VEGF level and increase NK cell level in in patients with non-squamous non-small cell lung cancer, have positive effect on cellular immune function, was worthy of clinical application.

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